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A33403 PCT USA -A - 072854.0114

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Potter et al.
Serial No.	:	09/633,697
Filed	:	August 7, 2000
For	:	HYDROXLATION ACTIVATED DRUG RELEASE

CLAIM FOR PRIORITY UNDER 35 U.S.C. § 119

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

A claim for priority is hereby made under the provisions of 35 U.S.C. § 119 for the above-identified continuation application of International Application PCT/GB99/00416 filed February 10,1999, claiming priority of Great Britain Application No. 9802957.2 filed December 12, 1998. These applications are listed in the declaration to the application, Serial No. 09/633,697, filed August 7, 2000. A certified copy of the Great Britain application is enclosed.

Respectfully submitted,

Ronald B. Hildreth
Patent Office Reg. No. 19,498

(212) 408-2544
Attorney for Applicants

Baker Botts L.L.P.
30 Rockefeller Plaza
New York NY 10112

09/633697

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

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Signed R. Mahoney

Dated 26 July 2000

Patents Form 1/77

Patents Act 1977
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12FEB98 E337756-1 D00443
P01/7700 25.00 - 9802957.2

EFFECTIVE FORM

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

M98/0082/GB

2. Patent application number
(The Patent Office will fill in this part)

12 FEB 1998 9802957.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

De Montfort University
The Gateway
Leicester
LE1 9BH

Patents ADP number *(if you know it)*

6741060001

If the applicant is a corporate body, give the country/state of its incorporation

Great Britain

4. Title of the invention

Hydroxylation Activated Drug Release

5. Name of your agent *(if you have one)*

McNeight & Lawrence

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Regent House
Heaton Lane
Stockport
Cheshire
SK4 1BS

Patents ADP number *(if you know it)*

0001115001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is NOT named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	9
Claim(s)	6
Abstract	
Drawing(s)	2

LSH

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature McNeight & Lawrence Date 12.02.98

12. Name and daytime telephone number of person to contact in the United Kingdom James A Robertson 0161 480 6394

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

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Patents Form 7/77

Patents Act 1977
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The Patent Office

23 NOV 1998

Statement of inventorship and of right to grant of a patent

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

M98/0082/GB

2. Patent application number
(if you know it)

9802957.2

3. Full name of the or of each applicant

De Montfort University

4. Title of the invention

Hydroxylation Activated Drug Release

5. State how the applicant(s) derived the right from the inventor(s) to be granted a patent

By virtue of employment

6. How many, if any, additional Patents Forms 7/77 are attached to this form?
(see note (c))

7. I/We believe that the person(s) named over the page *(and on any extra copies of this form)* is/are the inventor(s) of the invention which the above patent application relates to.

Signature McNeight & Lawrence

Date 20.11.98

8. Name and daytime telephone number of person to contact in the United Kingdom

James A Robertson 0161 480 6394

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there are more than three inventors, please write the names and addresses of the other inventors on the back of another Patents Form 7/77 and attach it to this form.

d) When an application does not declare any priority, or declares priority from an earlier UK application, you must provide enough copies of this form so that the Patent Office can send one to each inventor who is not an applicant.

e) Once you have filled in the form you must remember to sign and date it.

AIS5291

Enter the full names, addresses and postcodes of the inventors in the boxes and underline the surnames

POTTER, Gerald Andrew
22 Hamilton Street
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Patents ADP number *(if you know it)*: 75556425O1

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Patents ADP number *(if you know it)*: 75556590O1

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Patents ADP number *(if you know it)*: 75556070O1

Reminder

Have you signed the form?

DUPLICATE

Hydroxylation Activated Drug Release

The present invention concerns prodrugs whose enzymatic hydroxylation results in their activation by the release of a drug moiety. It particularly concerns anti-tumour prodrugs and those which are specifically activated by the hydroxylation activity of the enzyme CYP1B1.

Many conventional cytotoxic drugs are known (for example colchicine, esperimycin, taxol, daunomycin and staurosporin) which can be used for chemotherapeutic purposes. However, they typically suffer from the problem that they are generally cytotoxic and therefore may affect cells other than those which it is wished to target. This can be alleviated somewhat by using targetted drug delivery systems, for example direct injection to a site of tumourous tissue, or by e.g. binding the cytotoxic agent to antibody which specifically recognises an antigen displayed by cancerous cells. Alternatively, electromagnetic radiation may be used to cause chemical changes in an agent at a desired site in the body such that it becomes cytotoxic. However, all of these techniques have, to a greater or lesser extent, certain limitations and disadvantages.

It has been reported (Murray, G.I. *et al.*, 15 July 1997, Cancer Research, 57: 3026-3031) that the enzyme CYP1B1, a member of the cytochrome P450 family of xenobiotic metabolizing enzymes, is expressed at a high frequency in a range of human cancers including cancers of the breast, colon, lung, oesophagus, skin, lymph node, brain and testis, and that it is not detectable in normal tissues. This led to the conclusion (p. 3030, final sentence) that "...the expression of CYP1B1 in tumour cells provides a molecular target for the development of new anticancer drugs that could be selectively activated by the presence of CYP1B1 in tumour cells". It was also reported (p.3030,

column 1 lines 15-17) that CYP1B1 is capable of 4-hydroxylation of estradiol. No specific anticancer drugs were suggested.

The present inventors have now succeeded in creating a range of prodrugs having a "carrier" framework with a drug moiety conjugated to it (the prodrug other than the drug moiety is referred to below as "the rest of the prodrug") which have little or no cytotoxic effect when in their normal state, but whose hydroxylation (for example by CYP1B1) results in the release of the drug moiety. With CYP1B1 as the hydroxylating enzyme, this provides for a self-targetting drug delivery system in which a non-cytotoxic (or at leat negligibly cytotoxic) compound can be administered to a patient, for example in a systemic manner, the compound then being hydroxylated at the site of tumour cells (intratumoural hydroxylation) to release the drug which acts to kill or otherwise affect the tumour cells. The fact that CYP1B1 is not expressed by normal cells means that the hydroxylation of the prodrug only occurs at the site of tumour cells and therefore only tumour cells are affected, thus providing a self-targetting drug delivery system.

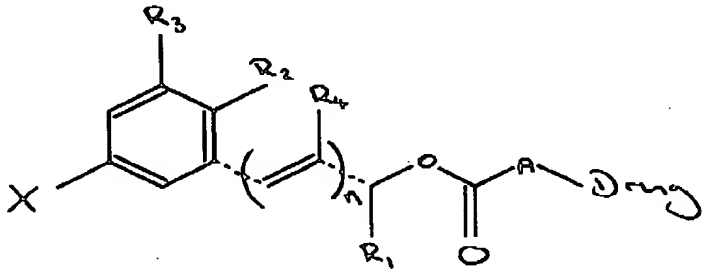
The prodrugs of the present invention have the distinct advantage of being useful in the treatment of tumours at any site in the body, meaning that even tumours which have undergone metastasis (which are not normally susceptible to site-specific therapies) may be treated, as well of course as primary and secondary tumours.

CYP1B1 has not yet been fully characterised, and it is therefore possible that tumour-specific isoforms of it may exist which possess the same catalytic properties. The prodrugs of the present invention may, of course, be used with such enzymes.

According to the present invention there is provided a prodrug having a drug moiety, the prodrug being activated by enzymatic hydroxylation to release the drug moiety.

Other enzymatically-activated prodrugs are known, for example those which release a drug moiety as the result of cleavage by a peptidase enzyme. However, nowhere has it been previously suggested that a prodrug could be activated by enzymatic hydroxylation.

A prodrug according to the present invention, being activated by enzymatic hydroxylation may have the formula (Z):



wherein:

X = H, OH, OMe or $N(CH_3)_2$; and

n = 0-6;

and:

R_1 = H, C_{1-4} lower alkyl, or together with R_2 forms part of a cycloalkyl group which may be further substituted to form part of a polycyclic cycloalkyl group, or with R_2 forms part of a steroidal carbon framework;

R_2 = H, C_{1-4} lower alkyl, or together with R_1 and/or R_3 forms part of a cycloalkyl, polycyclic cycloalkyl or steroidal carbon framework, or forms part of a polycyclic aromatic group by linkage to R_4;

R_3 = H, C_{1-4} lower alkyl or together with R_2 forms part of a cycloalkyl, polycyclic cycloalkyl or steroidal carbon framework; and

R_4 = H or is fused directly to the aromatic position designated by R_2

and either:

the drug moiety is derived from a drug having a free amino, hydroxyl or thiol group and which links it to the rest of the prodrug, such that A represents NH, NR (R=C_{1-4} lower alkyl), O or S; or

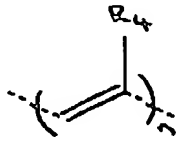
the drug moiety is derived from a drug having a carboxylate group, an ester linkage joining it to the rest of the prodrug and A being absent.

Enzymatic hydroxylation of the prodrugs of formula (Z) results in the transfer of electrons from the site of hydroxylation (for example the aromatic 4 position - see Figure 1) to the drug moiety, resulting in its release.

The prodrug may, for example, be an anti-tumour prodrug. The drug moiety may be cytotoxic or cytostatic, although of course it may be a moiety which has any other desired effect. Examples of classes of drug moiety include antimitotic agents, alkylating agents, antifolates, DNA-damaging agents and enzyme inhibitors. Specific examples of possible drug moieties include colchicine, esperimycin, taxol, daunomycin, staurosporin, and nitrogen mustard.

A possible nitrogen mustard is, for example, a para-hydroxy aniline mustard that is linked through the para-hydroxy group to the rest of the prodrug. In the case of nitrogen mustard prodrugs, the mustard function is itself activated only when the drug moiety is released from the prodrug.

The olefin linkage



may have a cis- or trans-geometry. It may be acyclic or cyclic. It may form part of an aromatic or polycyclic aromatic system.

The prodrug may be activated by CYP1B1. Thus a prodrug which releases a cytotoxic drug moiety upon hydroxylation by CYP1B1 may be used as a self-targetting anti-tumour drug, being activated at the site of a tumour by CYP1B1 and having no (or negligible) cytotoxicity in the rest of the body.

The linkage to the drug moiety from the rest of the prodrug may be from a hydroxyalkyl group in the prodrug *via* a carbamate, carbonate or thiocarbonate linker to an amino, hydroxy or thiol group in the drug moiety.

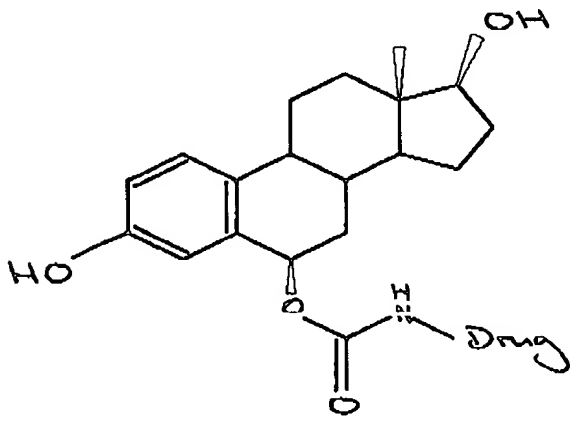
Using the strategy and prodrugs of the present invention, it is possible to link any desired drug moiety through a free amino, hydroxy or thiol group. The provision of a linker group comprising a carbamate, carbonate or thiocarbonate linker joining the drug moiety to the rest of the prodrug results in the release of carbon dioxide upon release of the drug moiety, making the reaction irreversible. Thus the hydroxylation of the prodrug may cause the release of the drug moiety and carbon dioxide.

A prodrug may have a steroid carbon framework. For example, it may be derived from estradiol.

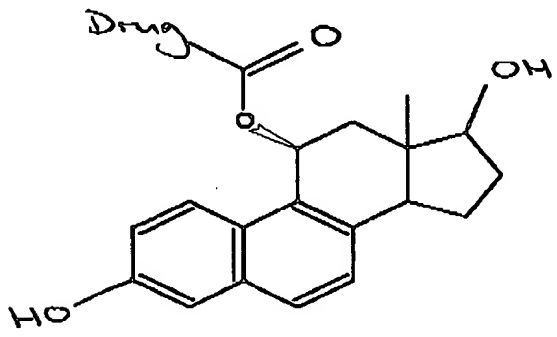
An example of a prodrug according to the present invention is the produg having the formula I, shown in Figure 1. It is an estradiol derivative and incorporates the drug moiety at the steroid 6-position. In this position, the 3-hydroxy group of estradiol does not provide the requisite electron release, but upon 4-hydroxylation the electron release from the 4-hydroxy group triggers electron transfer within the prodrug, resulting in the release of the drug moiety.

A prodrug according to the present invention may, for example, have the formula of any one of formulae (I) - (VII):

(I):



(II):



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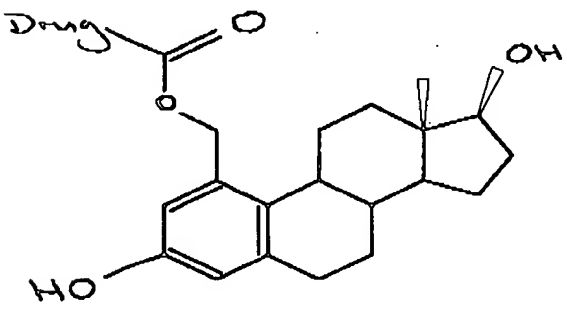

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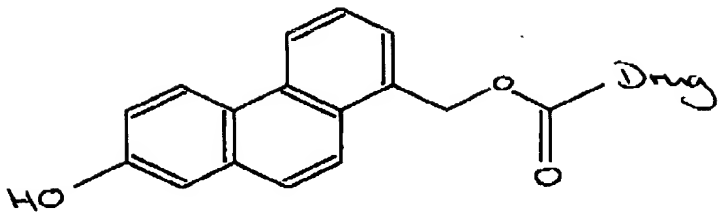

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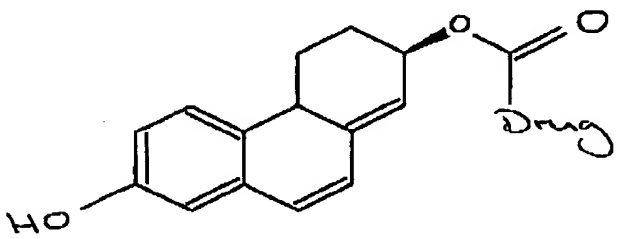

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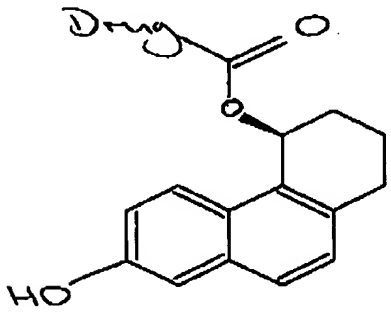
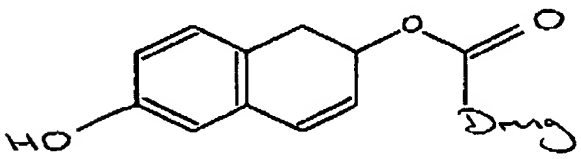


(VII):



Also provided according to the present invention is a prodrug according to the present invention for use in a method of treatment or diagnosis of the human or animal body, particularly the treatment or diagnosis of tumours.

Also provided according to the present invention is the use of a prodrug according to the present invention in the manufacture of a medicament for the treatment of tumours.

Also provided according to the present invention is a method of manufacture of a medicament for the treatment of a tumour, comprising the use of a prodrug according to the present invention.

Also provided according to the present invention is a method of treatment of a tumour in a patient, comprising administering to the patient a prodrug according to the present invention.

Methods of manufacture of medicaments are well known. For example a medicament may additionally comprise a pharmaceutically acceptable carrier, diluant or excipient (Reminton's Pharmaceutical Sciences and US Pharmacopeia, 1984, Mack Publishing Company, Easton, PA, USA).

The exact dose (i.e. a pharmaceutically acceptable dose) of prodrug to be administered to a patient may be readily determined by one skilled in the art, for example by the use of simple dose-response experiments.

Since prodrugs of the present invention may be specific to tumour cells, they may not only be used to treat tumours, but may also be used to determine whether or not a patient (or a sample taken from a patient) has tumour cells. For example, cell numbers in a sample may be assayed, as may the presence and quantity of the hydroxylated prodrug, thus providing for the diagnosis of the presence of tumour cells.

The invention will be further apparent from the following description, with reference to the several figures of the accompanying drawings, which show, by way of example only, forms of prodrug.

Of the figures:

Figure 1 shows the estradiol-derived prodrug having the formula (I), together with its 4-hydroxylation; and

Figure 2 shows the synthesis of an estradiol-colchicine produg. R is designated as representing H or a protecting group, for example an acetate group ($COCH_3$) or a benzyl group ($CH_2C_6H_5$).

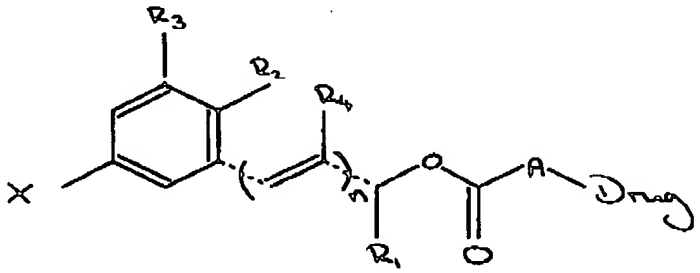
The synthesis of the estradiol-colchicine prodrug I is shown in Figure 2. The synthetic route uses estradiol as a starting material. The 6-oxo group is introduced by oxidation of estradiol with pyridinium chlorochromate to give 6-oxo estradiol. Thsi is then subjected to borohydride reduction to produce 6-hydroxy estradiol. Tthe desired cytotoxic agent is then coupled to the 6-hydroxy estradiol using triphosgene as coupling agent to provide the carbamate linked estradiol prodrug. In the synthesis of the prodrug, the R group is initially a protecting group (for example an acetate group). Once the final step (above) has been taken, the protecting groups are substituted with hydrogen to give the final prodrug product. The chemistry of protecting groups and their substitution is well known and will be readily apparent to one skilled in the art.

4-hydroxylation of the prodrug (Figure 1) results in electron transfer from the 4-hydroxy group, causing release of the drug moiety and carbon dioxide. The release of carbon dioxide makes the reaction irreversible.

CLAIMS

1. A prodrug having a drug moiety, the prodrug being activated by enzymatic hydroxylation to release the drug moiety.

2. A prodrug according to claim 1, being activated by enzymatic hydroxylation and having the formula (Z):



wherein:

X = H, OH, OMe or $N(CH_3)_2$; and

n = 0-6;

and:

R_1 = H, C_{1-4} lower alkyl, or together with R_2 forms part of a cycloalkyl group which may be further substituted to form part of a polycyclic cycloalkyl group, or with R_2 forms part of a steroidal carbon framework;

R_2 = H, C_{1-4} lower alkyl, or together with R_1 and/or R_3 forms part of a cycloalkyl, polycyclic cycloalkyl or steroidal carbon framework, or forms part of a polycyclic aromatic group by linkage to R_4;

R_3 = H, C_{1-4} lower alkyl or together with R_2 forms part of a cycloalkyl, polycyclic cycloalkyl or steroidal carbon framework; and

R_4 = H or is fused directly to the aromatic position designated by R_2

and either:

the drug moiety is derived from a drug having a free amino, hydroxyl or thiol group and which links it to the rest of the prodrug, such that A represents NH, NR (R=C_{1-4} lower alkyl), O or S; or

the drug moiety is derived from a drug having a carboxylate group, an ester linkage joining it to the rest of the prodrug and A being absent

3. A prodrug according to either one of claims 1 or 2, being an anti-tumour prodrug.

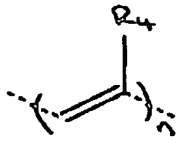
4. A prodrug according to any one of the precedign claims, the drug moiety being a cytotoxic or cytostatic agent.

5. A prodrug according to any one of the preceding claims, being activated by hydroxylation by CYP1B1.

6. A prodrug according to any one of the preceding claims, the drug moiety being an antimitotic agent, an alkylating agent, an antifolate, a DNA-damaging agent or an enzyme inhibitor.

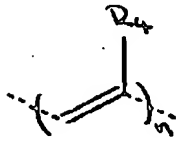
7. A prodrug according to claim 5, a cytotoxic drug moiety being selected from the group of colchicine, esperimycin, taxol, daunomycin, staurosporin, and nitrogen mustard.

8. A prodrug according to any one of the preceding claims, the olefin linkage



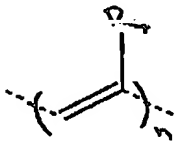
having a cis- or trans-geometry.

9. A prodrug according to any one of the preceding claims, the olefin linkage



being acyclic or cyclic.

10. A prodrug according to any one of the preceding claims, the olefin linkage



forming part of an aromatic or polycyclic aromatic system.

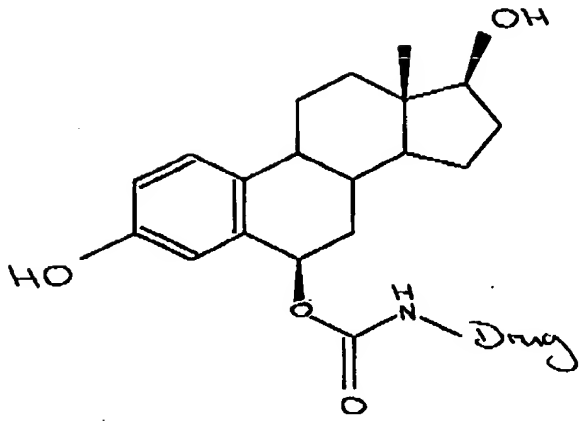
11. A prodrug according to any one of the preceding claims, the linkage to the drug moiety from the rest of the prodrug being from a hydroxyalkyl group in the prodrug *via* a carbamate, carbonate or thiocarbonate linker to an amino, hydroxy or thiol group in the drug moiety.

12. A prodrug according to any one of the preceding claims, having a steroid carbon framework.

13. A prodrug according to any one of the preceding claims, being derived from estradiol.

14. A prodrug according to any one of the preceding claims, having the formula of any one of formulae (I) - (VII):

(I):



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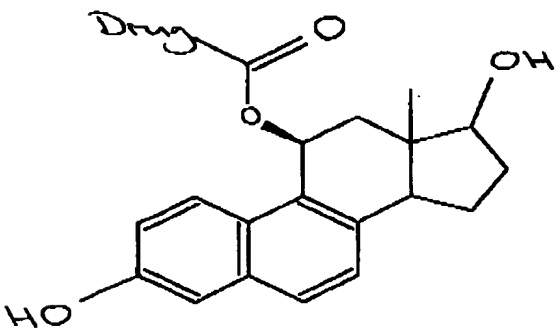

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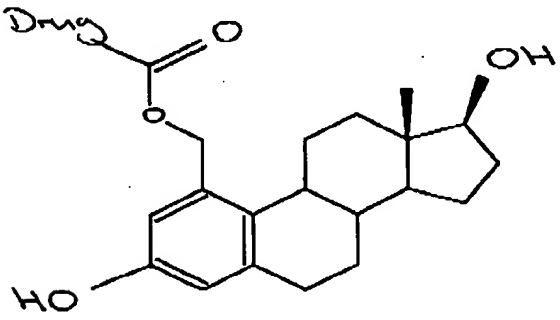

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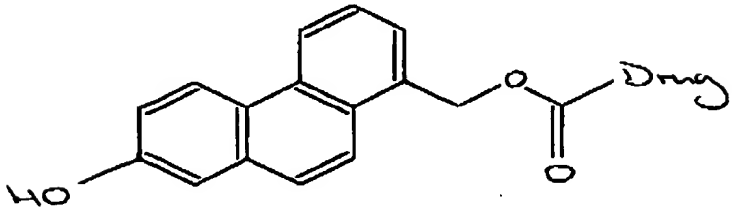
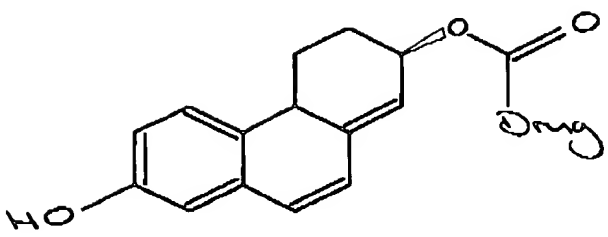
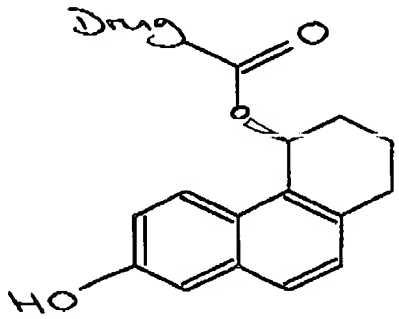


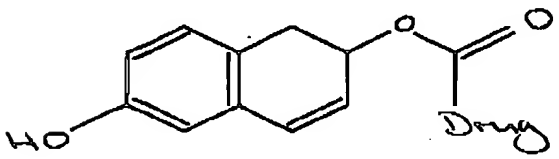
(V):



(VI):



(VII):



15. A prodrug according to any one of the preceding claims, its hydroxylation causing the release of the drug moiety and carbon dioxide.

16. A prodrug according to any one of the preceding claims for use in a method of treatment or diagnosis of the human or animal body.

17. The use of a prodrug according to any one of claims 1-15 in the manufacture of a medicament for the treatment of tumours.

18. A method of manufacture of a medicament for the treatment of tumours, comprising the use of a prodrug according to any one of caims 1-15.

19. A method of treatment of a tumour in a patient, comprising administering to the patient a prodrug according to any one of claims 1-15.

Figure 1

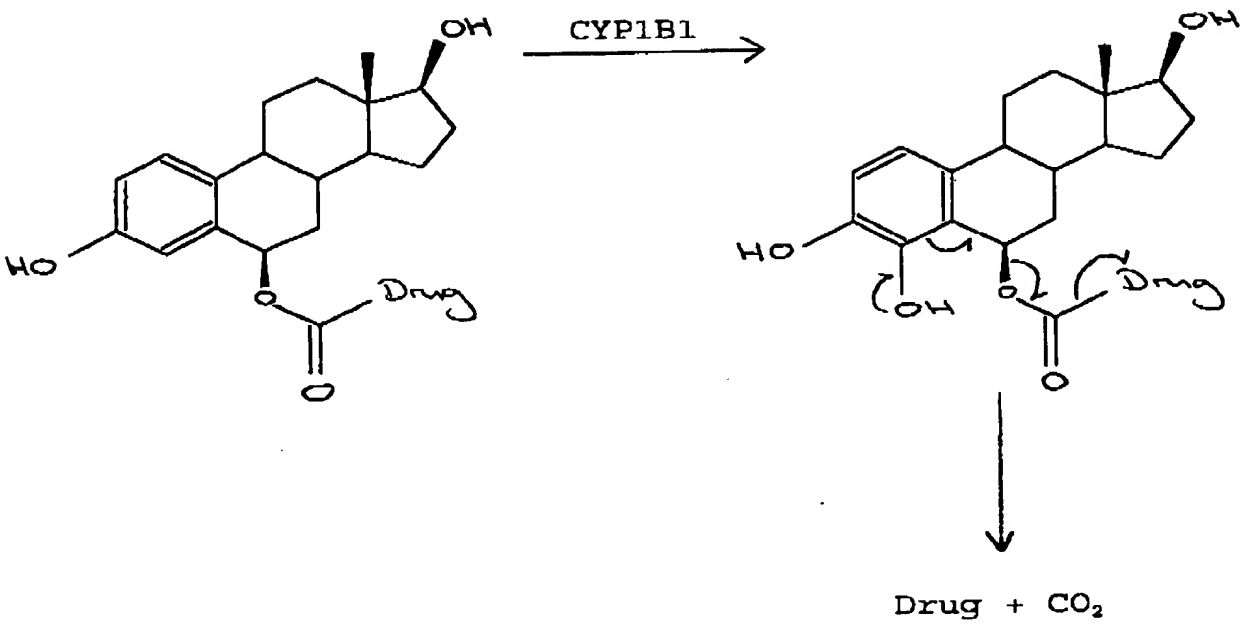
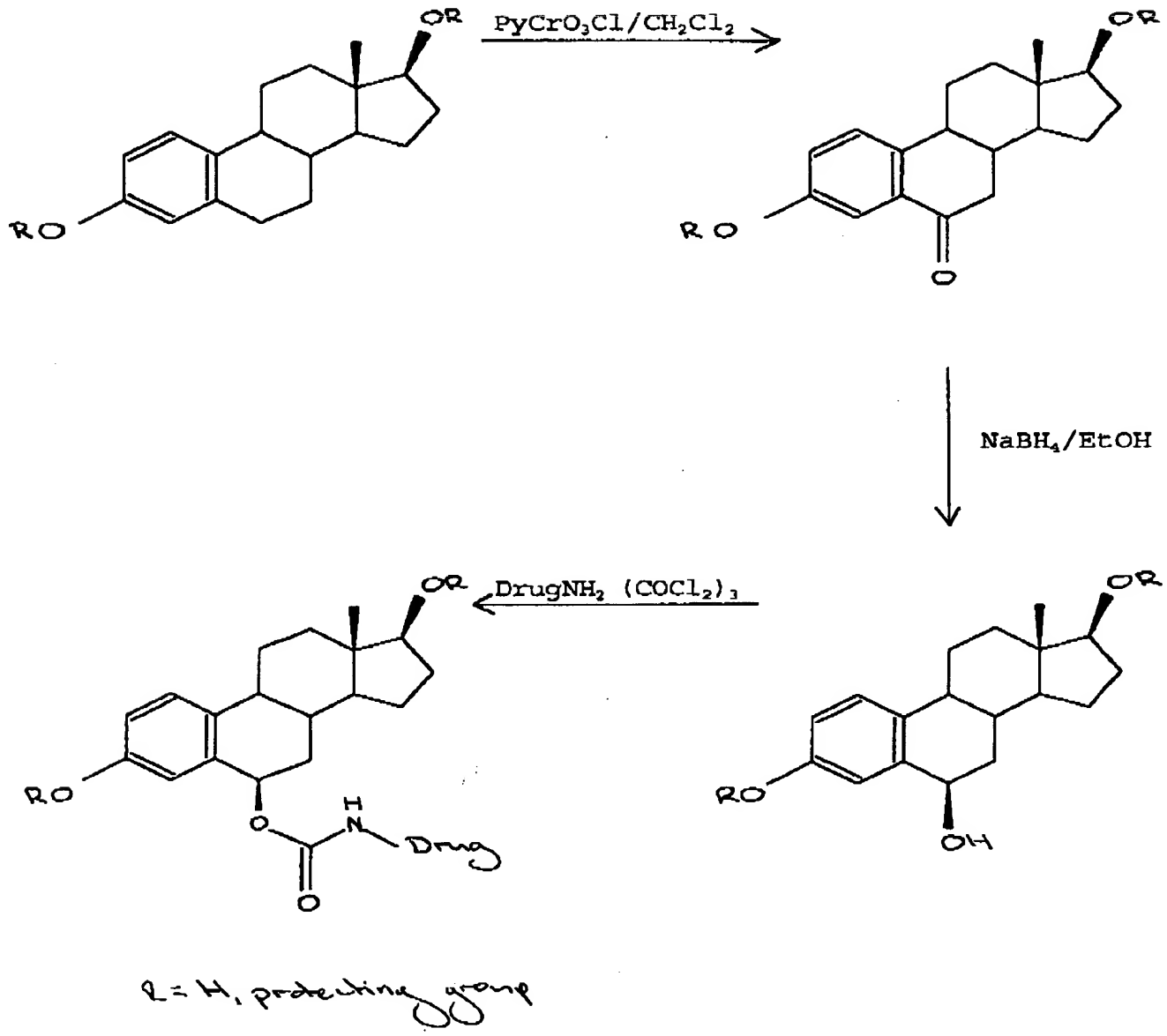


Figure 2



R = H, protecting group